Protocol for RNA annealing/duplex formation

General Handling instructions

RNA-oligonucleotides produced and delivered by metabion **are deprotected and HPLC purified by default**. Although our oligonucleotides are RNAse free, RNA is highly susceptible to degradation by exogenous and ubiquitous RNAses introduced during handling. Therefore, it is essential that all handling steps are performed under RNAse free conditions. RNA oligonucleotides should not be handled without gloves. RNAse free reagents, barrier pipette tips and tubes should be used. Dry RNA oligonucleotides can be safely stored at –20 °C for up to 6 months.

Annealing of RNA/Duplex Formation

While some manufacturers state that re-annealing of RNA is generally not necessary we recommend to anneal RNA duplexes prior to your experiments using a suitable protocol (see below).

If you ordered two single strand (ss) RNAs, separately dissolve both RNA oligonucleotides at a convenient stock concentration, e.g. at 100 µM (recommended), in RNAse free water (pH >4<9). We recommend to prepare aliquots and store those, which are not used immediately at -20 °C.

- Separately dilute one aliquot per RNA oligonucleotide to a final concentration of 50µM using RNAse free water.
- Mix 40 µl of each RNA oligonucleotide solution and add 20 µl of 5 x **Annealing Buffer***, arriving at a final volume of 100 µL, and a final RNA duplex concentration of 20 µM.
- Incubate the solution for 1 min at 90-95 °C and gradually/slowly cool down to room temperature (over a period of no less than 30 min). Store at 4 °C or on ice until ready to use. We recommend to process and use this re-annealed working solution within 24 hours.

If you ordered an RNA duplex, dissolve the delivered pellet in 1 x **Annealing Buffer*** at a convenient stock concentration, e.g. 100µM. We recommend to prepare aliquots and store those, which are not used immediately, at -20 °C.

- For duplex formation, dilute one dsRNA aliquot to the concentration needed for your experiment (e.g. 20 µM) using 1 x Annealing buffer.
- Incubate the solution for 1 min at 90-95 °C and gradually/slowly cool down to room temperature (over a period of no less than 30 min). Store at 4 °C or on ice until ready to use. We recommend to process and use this re-annealed working solution within 24 hours.

Annealed RNA duplexes can be safely stored at –20 °C. Do not freeze-thaw more than 5 times. We recommend to perform the re-annealing procedure described above before using thawed duplex aliquots.

***Annealing buffer:**

5 x Annealing buffer concentration: 300 mM KCl, 30 mM HEPES-pH 7.5, 1.0 mM $MgCl_2$

1 x Annealing buffer concentration: 60 mM KCl, 6 mM HEPES-pH 7.5, 0.2 mM $MgCl_2$

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References

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